The developed TaqMan real-time PCR assay for EHV-5 detected and quantified multiple currently circulating isolates of EHV-5, while not detecting DNA from other equine herpesviruses, including equine herpesvirus 2, a close homolog of EHV-5. Using TaqMan real-time PCR, we were also able to quantify viral copy number in EHV-5 inoculated ERECs over time and show significantly increased viral copies on days 5 and 6 post-inoculation that declined sharply by day 12 post-inoculation. Quantification was not possible using conventional PCR. In conclusion, the developed TaqMan real-time PCR assay is a rapid, specific, and sensitive test that can be used to more accurately describe initial events following EHV-5 infection and begin to understand viral pathogenesis and establishment of latency. Furthermore, this assay will be useful in future *in vivo* investigations to study the epidemiology and pathogenesis of EHV-5 in its host.

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Validation of an easy handling sample preparation and a triplex real-time polymerase chain reaction for rapid detection of contagious equine metritis organisms

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Taylorella equigenitalis is the causative agent of contagious equine metritis (CEM). This venereal disease often originates from a symptomless carrier and can spread rapidly in mares and stallions. Isolation and identification of *T. equigenitalis* is difficult due to a low growth rate, specific culture requirement and limited differentiation from *Taylorella asinigenitalis*. Differentiation of *Taylorella* species is essential as only *T. equigenitalis* has been demonstrate to infect horses. Recovery of two others organisms implicated in equine metritis, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* is also important. Rapid information about the bacterial load measured by PCR method could be a valuable tool for diagnosis and management of metritis. Most PCR-based diagnostics are still considered time-consuming due to disparate purification, amplification, and detection steps. Our objective was to significantly reduce sample preparation and assay time. We have develop and evaluate a detection method including a rapid preparation of the sample combined to two real-time PCR assays for the detection of *T. equigenitalis* and *T. asinigenitalis* in one hand, and *K. pneumoniae* and *P. aeruginosa* in the other hand. Each PCR targets a gene specific for each pathogen and contains an exogenous internal control. The validation of these two assays followed the AFNOR guideline NF U47-600. 1) The analytical specificity, sensitivity and limit of detection (LOD_{PCR}) were defined for each real time PCR assays. The analytical specificity of the PCR test was assessed on 99 different species that could be recovered from clinical samples as well as 24 strains of *T. equigenitalis*, 14 of *T. asinigenitalis*, 33 of *K. pneumoniae* and 16 of *P. aeruginosa*. LOD_{PCR} was determined to be 5 genome equivalent (GE)/PCR reaction for *T. equigenitalis* and 20 GE/PCR reaction for the others pathogens. 2) The whole analytical process, from the lysis of the sample to the PCR analysis, was characterised. Whole analytical process simply consists in lysis of Amies charcoal swab emulsion directly analysed by PCR. LOD_{Method} was determined as 100 and 7500 UFC/mL according to the pathogen. Diagnostic sensitivity and specificity of our solution are estimated from 500 Amies charcoal swabs received directly from the field. CEM organisms status were defined by culture which is gold standard method. The sensitivity of the overall method is 100% and the specificity is greater than 92%. In conclusion, by eliminating sample purification before PCR analysis, we can provide a simple-to-use method for the detection of pathogens implicated in equine metritis and an alternative to culture during the breeding season.
*AFNOR NF U47-600 norm: Requirements and recommendations for the implementation, the development and the validation of veterinary PCR for animal health analysis methods.

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Dual antigen ELISA to differentiate infection from vaccine antibody responses to *Leptospira* spp.

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Research in our laboratory and elsewhere has identified a number of strongly immunogenic proteins of Leptospira interrogans serovar Pomona type kennewicki (Lk) that are up-regulated in infected horses but are undetectable or expressed only in trace amounts on cultured organisms. Examples of these proteins are Sph2 (Lk73.5), LigA and its truncate Lk90, Hsp15 and LipL45 (Qlp42). In contrast, the immunogenic LipL32 is expressed abundantly on cultured L. interrogans serovar Pomona type kennewicki and other serovars and elicits serum antibody response in infected horses. Horses vaccinated with heat-killed cultures of serovar Pomona type kennewicki would therefore be predicted to make serum antibody responses to LipL32 and Lk sonicate, but not to Sph2, LigA/Lk90, Hsp15 and LipL45. The aim of this study was to develop an ELISA based on LipL32 or Lk sonicate and one or more of the host-induced proteins with value in differentiating vaccine serum antibody responses from those induced by infection. Recombinant Sph2, LigA, Lk90, Hsp15, LipL45 and LipL32 proteins of L. interrogans serovar Pomona type kennewicki were expressed and purified and antibody levels (IgG) specific for these proteins were assayed in sera from horses (a) before and after immunization with killed vaccine + adjuvant and b) infected naturally with L. interrogans serovar Pomona type kennewicki. Sera diluted 1:200 were assayed in triplicate using optimum concentrations of recombinant proteins. Bound IgG was detected using HRP-Protein G conjugate. Based on antibody responses of horses, reactive proteins formed 3 groups, group 1 (Sph2, Lk90 and LigA) that reacted strongly with convalescent sera but not with pre and post vaccine sera; group 2 (LipL45 and Hsp15) that reacted moderately with convalescent sera and at very low reactivity with pre and post vaccine sera and group 3 (Lk sonicate and LipL32) that reacted strongly with convalescent as well as post vaccine sera but unreactive with pre vaccine sera. Antibodies to Group 1 proteins were significantly higher ($p \leq 0.01$) in convalescent sera than in pre and post vaccine sera. The experimental vaccine based on L. kennewicki cultured in vitro theoretically lacks expression of the host-induced proteins Sph2, LigA and its truncate Lk90, LipL45 and Hsp15. As expected, vaccine sera failed to react with these proteins. Significantly higher levels of antibody against constitutively expressed LipL32 and Lk sonicate in post vaccine sera as compared to pre vaccine sera indicate horses responded well to the experimental vaccine. A dual antigen ELISA based on Lk sonicate or LipL32 combined with host-induced Sph2 and Lk90 proteins will be valuable in differentiating responses of infected from vaccinated animals (DIVA). However, differentiation of horses with recent previous exposure to Leptospira that are subsequently vaccinated may not be possible.